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THE GERMICIDAL POWER OF ANTISEPTIC OILS AND OF SUBSTANCES DISSOLVED IN OIL *

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It has been known definitely since the time of Koch that certain essential oils have considerable disinfectant value. It has been assumed since his time also that substances which have disinfectant value when in watery solution are largely rendered inactive by solution in oil. This assumption arose as a wide generalization from what was actually observed by Koch in only one case, namely, that of phenol in vegetable oils, such as olive oil or cotton seed oil. In this case the validity of the observation is undoubted, but there have been a very few practitioners who have believed that in spite of the test tube indications the best way to apply phenol as a wound disinfectant is in solution in vegetable oil. The recent application by Dakin[7] and his associates, of solutions of a water-insoluble oil-soluble substance (dichloramin-T) to the treatment of wounds indicates that from the practical point of view the question of the usefulness of disinfectants in oil is by no means closed. As a consequence of these general considerations, I undertook to study these questions anew.

The recognized methods of determining quantitatively the antiseptic value of substances presuppose water solubility. The immiscibility of water and oil prevents the use of broth culture (or the suspension of bacteria in salt solution) in the testing of an oily substance, or of material which has been in contact with an oil. It is doubtless for this reason — the physical difficulty of testing by standard methods — that the many new antiseptic oils and oil-soluble antiseptics are accepted solely on the basis of clinical experience and not on that of known germicidal power. The value of such oils has not up to the present been expressed in terms of a known standard, comparable to the "phenol coefficient" of water-soluble antiseptics.

Face to face with the necessity of deciding accurately the antiseptic power of a large number of oils the need for a preliminary investiga-

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Received for publication Dec. 21, 1918.

* An investigation of disinfectants including certain oily solutions under the direction of Drs. Paul A. Lewis and A. N. Richards, made at the request of the medical committee of the Council of National Defense and carried out in the John Herr Musser Department of Research Medicine, University of Pennsylvania.

tion bearing on the possibility of determining the disinfectant power of any oil or substance dissolved in oil with sufficient occuracy to permit of quantitative expression became apparent.

The literature of this subject is scanty and shows that the favorite test, under these circumstances, has been that of the silk thread with dried anthrax spores. Koch[1] studied by this method the bactericidal properties of the essential oils, using them in full strength and expressing their power in terms of time. The killing times for bacteria of oils, such as oil of turpentine and eucalyptol, have been tested by Laubenheimer[2] in like manner.

Other observations are those of Kronig and Paul,[3] Cadéac and Meunier,[4] and Chamberland.[5]

My preliminary experiments were made with dried anthrax spores on silk threads, from which the oil was removed, after the test period, by washing with various substances capable of dissolving the oil, as alcohol, ether, chloroform, xylol, etc., but the results were so irregular and conflicting that this method was discarded as unsatisfactory.

I then approached the matter by planting B. typhosus on the surface of rather dry slants of nutrient agar, covering the growth with the oil or oily solution to be tested. After a suitable period of exposure the oil was poured off, the tubes were filled with sterile salt solution to float up the remaining oil droplets; this was again poured off and the viability of the remaining growth was tested by transplantation to fresh medium. Broth was chosen as a medium for this test transplantation with the idea that any oil remaining with the bacterial mass would be more certainly separated than would be true if agar slants were again used.

In the standard methods for determining the efficiency of disinfectants, known generally as either the Rideal-Walker or the Hygienic Laboratory test, the final expression is based on the concentration required to kill a definite number of bacteria in a certain time. The time for convenience is made very short, 5 minutes and 15 minutes being the accepted periods.

It was obvious that the test in hand could not be worked with these periods. The uneven thickness of the growth involving irregularities of penetration and the loss of time in washing off the oil must necessarily be compensated for by extending the time of exposure. Twenty-four hours seemed most convenient and was found in practice to give consistent results.

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[1] Mittheil. a. d. K. Gesundheitsamte, 1881, 1, p. 234.

[2] Allgemeine Bakteriologie und Sterilisationslehre für Aerzte und Pharmazeuten, 1915.

[3] Ztschr. f. Hyg., 1897, 25, p. 5.

[4] Ann. de l'Inst. Pasteur, 1889, 3, p. 317.

[5] Ann. de l'Inst. Pasteur, 1887, 1, p. 153.

As a preliminary, experiments were made to determine the influence of the age, extent, and thickness of the culture upon the concentration required to kill. The time of exposure was 24 hours, and the temperature 37.5 C. Cultures were prepared of certain well defined types, as follows:

In type 1 the growth was a narrow smear with the base extending low enough to touch any water of condensation that might be in the tube. In type 2 the growths were mere spots. In type 3 the growth was smeared widely at the bottom in order to come in contact with any water of condensation which, collected at the base of the tube, might interfere with results. In type 4 the growth was kept well above any such water. Type 5 was similar to type 4 except that the surface of the agar was purposely cut deeply. In types 1 to 4 the surface of the mediums was not intentionally broken.

About 20 tubes of each type were planted, and after 24-48 hours' growth the tubes were filled with paraffin oil containing various percentages of phenol.

Of the 48-hour cultures none were killed by 0.2%, but all were killed by 0.6% phenol. The general results indicate that the killing concentration varies within a narrow range, between 0.2 and 0.6%. Likewise in the 24-hour cultures, in no case did 0.2% kill, but in about half of the tubes 0.4% killed, and in all cases 0.6% killed.

The most resistant cultures were those in which the surface had been intentionally wounded, the growth being therefore rather deep, and presumably protected from the action of the disinfectant.

When growth was obtained with 0.4% it occurred only in types in which the agar surface was wounded. In all other experiments in which the surface was not wounded death occurred at 0.4%.

These experiments therefore show that differences in the characteristics of the growths do not influence the action of the disinfectants if the surface is not injured. Control tubes in which paraffin oil was allowed to cover the growth for one day and two days gave growth after transplantation showing that the oil alone does not kill.

With this evidence at hand the next step was to determine the most suitable type of growth.

Over 100 tubes were planted of two types, and cultures of 24 hours were used. The two types were a simple streak, well above the bottom of the tube (type 4), and two dots also well above the water of condensation (type 2).

The three substances tested in this way were phenol, paracresol, and guaiacol. The results are shown in Table 1.

The minimal killing concentration of phenol is 0.4%; of paracresol, 0.2-0.3%, and of guaiacol 0.8-1%.

TABLE 1

TESTS MADE WITH THREE SUBSTANCES WITH TWO TYPES OF GROWTH

Substance Tested	Type of Growth	Dilution of Disinfectant in Paraffin Oil									
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Guaiacol	IV*		++ ++ ++		++ ++ ++		++ ++ ++		− − ∓		− − −
	II		++ ++ ++		++ ++ ++		++ ++ ++		− ++ ++		− − −
Paracresol	IV	++ ++ ++	− − −	− − −	− − −		− − −				
	II	++ ++ ++	++ − ++	− − ∓	− − −		− − −				
Phenol	IV		++ ++	− − −	− − −	− − −	− − −				
	II		++ − ++	− − −	− − −	− − −	− − −				

* In each test three tubes were used. + = growth; — = no growth.

It will be noted that the irregular results with paracresol and guaiacol occurred only in type 4 cultures, in which it is possible the surface of the mediums was punctured at time of inoculation. In all type 2 cultures the results were uniform.

With this experience a technic was adopted as follows: Agar slants, well drained of water of condensation, were inoculated with B. typhosus over an area not more than one centimeter and well above the middle of the tube. They were allowed to incubate 24 hours. The oily solutions were then poured over these so as to entirely cover the agar and the tubes allowed to stand 24 hours in the incubator at 37.5 C. The oily solutions were then poured off and the tubes twice washed out carefully with sterile salt solution. For this purpose an inverted wash bottle with a long pipe and a spring clamp on the tubing above a glass beak served the purpose well. The growth was thus thoroughly washed on the surface of the agar and a transplant made to a tube containing 8 c.c. standard broth. Readings were made of these tubes after 24 and 48 hours' incubation.

For all purposes the 24 hour reading is satisfactory. In the total number of experiments only 4 tubes found negative at 24 hours became positive at 48 hours.

In order to determine whether phenol, dissolved in paraffin oil, would serve well as an arbitrary standard, in the testing of oily disinfectants, as it has in the testing of watery solutions, the following tests were made.

Solutions of phenol, paracresol, and guaiacol were made in paraffin oil and in water and tested simultaneously. The testing of the three substances in oil was according to methods previously outlined. In like manner the watery solutions were poured over agar tubes and tested as were the oil substances. Lastly, determinations of the phenol coefficients of the three substances were made by the Anderson-McClintock[6] method. The results follow:

			Coefficients
In paraffin oil	phenol	kills at 0.4%; not at 0.3%	= 1.00
	guaiacol	kills at 0.8%; not at 0.7%	= 0.475
	paracresol	kills at 0.3%; not at 0.2%	= 1.55
In water	phenol	kills at 1.0%; not at 0.9%	= 1.00
	guaiacol	kills at 1.4%; not at 1.3%	= 0.69
	paracresol	kills at 0.6%; not at 0.5%	= 1.73
By the Anderson-McClintock method	phenol	coefficient	= 1.00
	guaiacol	coefficient	= 0.75
	paracresol	coefficient	= 1.55

Or, more clearly expressed:

The coefficients of	By Anderson-McClintock method	In paraffin oil	In water
Phenol	1.00	1.00	1.00
Guaiacol	0.75	0.475	0.69
Paracresol	1.55	1.55	1.73

These results seem to justify the use of the typhoid agar method in the testing of oils.

With the method thus elaborated, a series of experiments were made, many of which were repeated, to determine the value of certain disinfectants in oil and of certain proprietary oily disinfectants. Among various oils tested as a menstruum were Diamond Oil, Eclipse White Spindle Oil, and Rose White Oil brands of mineral oil furnished by the Atlantic Refining Company. Experiments showed that the action of phenol in all these oils is the same. The difference in the composition of the oil has no apparent effect; 0.4% phenol kills in all cases, 0.3% is the border line, and 0.2% fails to kill in all cases. Control tubes in which the oils alone were used, gave growth in all instances.

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[6] The Determination of the Phenol Coefficient of Some Commercial Disinfectants, Hygienic Laboratory Bulletin 82, 1912.

Substance Tested	Lethal Point, %	May Kill, %	Does Not Kill, %	Coefficient
Phenol in paraffin oil	0.4	0.3	0.2	1.00
Iodin in paraffin oil	0.4	0.3	0.2	1.00
Phenol in cotton seed oil	2.5	2.0	1.0	0.16
Paracresol in paraffin oil	0.25	0.2	0.1	1.55
Guaiacol in paraffin oil	0.9	...	0.8	0.44
Proprietary preparation No. 1 in paraffin oil	10.0	...	5.0	0.04
Proprietary preparation No. 2 in paraffin oil	6.0	5.0	4.0	0.06
Proprietary preparation No. 3 in paraffin oil	10.0	7.5	5.0	0.04
Proprietary preparation No. 4 in paraffin oil	2.0	...	0.5	0.2
Dichloramin-T in 10% chlorinated eucalyptol in chlorinated paraffin oil	0.05	...	0.03	8.00
Dichloramin-T in plain paraffin oil	0.4	...	0.2	1.00

In all these experiments controls were used in which plain paraffin oil was poured over the agar slants. In all such, growth was obtained after 24 hours.

Experiments with menthol, camphor and turpentine resin showed that these did not kill in the highest concentrations that could be obtained.

As a preliminary to a series of tests of dichloramin-T a few experiments were made on the bactericidal properties of paraffin oil, eucalyptol, chlorinated paraffin oil and chlorinated eucalyptol, the results of which follow:

Paraffin oil alone, growth after 24 and 48 hours exposure.

Eucalyptol plain, growth after 24 and 48 hours exposure.

Ten per cent. eucalyptol in chlorinated paraffin oil, growth after 24 hours, kills after 48 hours 'exposure.

Eucalyptol partially chlorinated, kills in 24 hours (growth in 8 hours).

Eucalyptol completely chlorinated, kills in 8 hours.

Dichloramin-T was then dissolved in chlorinated paraffin oil containing 10% chlorinated eucalyptol. Dilutions were always made with a 10% chlorinated eucalyptol in chlorinated paraffin oil, in order to keep the percentage of eucalyptol constant. Two sets of observations were made, and it was found that dichloramin-T in 0.05% kills, but not always 0.03%.

In this experiment, tubes covered with 10% eucalyptol dissolved in chlorinated paraffin oil but without dichloramin-T showed growth. This growth, however, was more scanty than that obtained with chlorinated paraffin oil alone.

In other tests in which dichloramin-T was added to plain paraffin oil, no growth was obtained at 0.4 and good growth at 0.3, giving the nonchlorinated combination a coefficient of 1, but reinforced with chlorinated paraffin oil a coefficient of 8.

In a final set of tests an attempt was made to reduce time of exposure. It is, of course, probable that a chlorin-containing substance as dichloramin-T in high dilution might exert its action in a shorter time than 24 hours and hence in determining the minimal killing concentration of such a substance a 24 hours' test would be inadequate. A comparison of the time reaction of dichloramin-T and phenol is presented in table 2 and shows that this is the case. In 6 hours the dichloramin has exerted its full action while the phenol required over 12 hours. The coefficient of dichloramin-T would therefore be so much the higher.

TABLE 2

TIME EXPERIMENT WITH PHENOL AND DICHLORAMIN-T IN PARAFFIN OIL

	Phenol					Dichloramin-T	
	0.6	0.5	0.4	0.3	0.2	0.06	0.05
3 hours		++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
6 hours	— —	— —	++ ++	++ ++	++ ++	— —	— —
12 hours	— —	— —	— —	++ ++	++ ++	— —	— —
24 hours	— —	— —	— —	— ++	++ ++	— —	— —

Despite these results we believe it preferable in routine work to base results on the minimal killing concentration at 24 hours' exposure.

CONCLUSIONS

A method which may provisionally be regarded as satisfactory for determining quantitatively the germicidal power of antiseptic oils and substances dissolved in oil, has been developed.

Phenol dissolved in mineral oil has germicidal value approaching that of its value in water (under the limited conditions of our test) and can serve as arbitrary standard for comparison when testing the activity of other oils and oil-soluble substances.

The method as so far developed is defective in that any advantage which a substance might possess in the shape of rapidity of action is largely masked by the 24-hour period of exposure used. Our work indicates that consideration given to this factor may lead to a limited degree of improvement in the method as here described.